

Invited Review Article

Role of airway epithelial barrier dysfunction in pathogenesis of asthma



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AJC, apical junctional complex; ALI, air-liquid interface; CDHR3, cadherin-related family member 3; EGF, epithelial growth factor; GSK-3 β , glycogen synthase kinase 3; KIF3A, kinesin family member 3A; MyD88, myeloid differentiation primary response gene 88; Nrf2, NF-E2-related factor 2; PAR2, protease-activated receptor-2; PCDH1, protocadherin 1; START, steroid-acute regulatory protein-related lipid transport; Stard, START domain; ST2, suppression of tumorigenicity 2; TRIF, TIR-domain-containing adapter-inducing interferon- β ; TSLP, thymic stromal lymphopoietin; STAT5, signal transducer and activator of transcription 5

ABSTRACT

Bronchial asthma is characterized by persistent cough, increased sputum, and repeated wheezing. The pathophysiology underlying these symptoms is the hyper-responsiveness of the airway along with chronic airway inflammation. Repeated injury, repair, and regeneration of the airway epithelium following exposure to environmental factors and inflammation results in histological changes and functional abnormalities in the airway mucosal epithelium; such changes are believed to have a significant association with the pathophysiology of asthma. Damage to the barrier functions of the airway epithelium enhances mucosal permeability of foreign substances in the airway epithelium of patients with asthma. Thus, epithelial barrier fragility is closely involved in releasing epithelial cytokines (e.g., TSLP, IL-25, and IL-33) because of the activation of airway epithelial cells, dendritic cells, and innate group 2 innate lymphoid cells (ILC2). Functional abnormalities of the airway epithelial cells along with the activation of dendritic cells, Th2 cells, and ILC2 form a single immunopathological unit that is considered to cause allergic airway inflammation. Here we use the latest published literature to discuss the potential pathological mechanisms regarding the onset and progressive severity of asthma with regard to the disruption of the airway epithelial function.

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Introduction

The airway epithelium is located at the interface between the internal and external environment and has long been an area of interest as it possibly plays an important role in the onset mechanism of asthma. Airway epithelial cells play an important role in innate immune functions in the lungs. Airway epithelial cells also

exhibit the characteristics of mucociliary cells and physically remove pathogens via a process known as the mucociliary escalator, which involves the trapping of pathogens in the mucus produced in airways under inflammatory conditions and removing the mucus via the movement of cilia present on epithelial cells. Moreover, the production of chemokines and cytokines mobilizes inflammatory cells, and their activation aids in removing microbes.^{1–3} However, excessive induction of this protective mechanism that is used to prevent infection may trigger the onset of chronic airway inflammation associated with asthma. It has recently become clear that epithelium-derived cytokines that promote the Th2 immune response and that are released because of injured and activated airway epithelial cells play a role in allergic

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inflammation, and the importance of the activation of airway epithelial cells in the pathophysiology of asthma has also been emphasized.⁴

Airway epithelial cells, which form a physical barrier against the external environment, comprise the apical junctional complex (AJC), formed in the junction between adjacent cells. AJC comprises tight junctions and adherens junctions, which form cellular junctions between similar and dissimilar cells.^{5–7} The tight junction is an intercellular junction between the apexes of cells, whereas the adherens junction functions as a barrier that regulates the passage of water-soluble substances and ions by creating a tight bond between the cell membranes of adjacent cells. In addition to their role as an intercellular binding mechanism, epithelial tight junctions and adherens junctions play an important role in establishing cell polarity.^{4–6} AJC dysfunction of the airway epithelia has been recently identified in patients with asthma, suggesting that AJC is important in the pathophysiology of asthma. This type of damage to the epithelial barrier allows inhaled allergens to infiltrate the submucosa of the airway and prevents the complete repair of epithelial cells and excessively activates the epithelial cell signal transduction cascade, which is considered to activate immune cells present in the submucosa.^{7,8} Therefore, disrupting the regulatory function of the airway epithelial barrier has recently been used as an important checkpoint for immunostimulation associated with asthma. Here we discuss the mechanism by which airway barrier function is disrupted in association with allergic airway inflammation and provide an overview of the disruption of the epithelial barrier and the resulting exacerbation of airway inflammation.

Disruption of the epithelial barrier owing to airway inflammation

Various environmental factors reportedly affect the tight junction barrier. Wan *et al.*^{9–11} reported that *Der p1*, a mite allergen closely associated with the onset of allergic inflammation, disrupts the tight junction directly through protease activation and indirectly through protease-activated receptor-2 (PAR2). These effects accelerate epithelial permeability, making it easy for allergens to infiltrate the airway submucosa. It was subsequently discovered that in addition to *Der p1*, protease activation caused by other mite and pollen allergens also disrupt the epithelial barrier.¹¹ Trypsin released from degranulated mast cells in response to allergen stimulation disrupts epithelial tight junctions by activating PAR2, which accelerates epithelial permeability.^{10,12–14} Moreover, multiple studies have found that cigarette smoke accelerates epithelial permeability by inducing reactive oxygen species.^{15–18} Among the factors associated with the onset and exacerbation of bronchial asthma, the presence of the respiratory syncytial virus or rhinovirus in the airway and infection of the airway epithelial cells by these viruses is known to disrupt tight junctions and promote airway epithelium permeability.^{19–24}

Cytokines reportedly alter tight junction functions. The proinflammatory cytokines, TNF- α and IFN- γ , disrupt the airway epithelial barrier,^{25–27} and the same results were obtained for the Th2 cytokines, IL-4 and IL-13.^{28,29} A study of the tight junction proteins in airway epithelial cells obtained via brushings from 67 patients with asthma and 42 healthy individuals reported that claudin-18 expressions were decreased in patients with asthma.³⁰ Claudin proteins determine the permeability of tight junctions. There are several dozen proteins in the claudin family, and their expression patterns differ according to the type of epithelial cell.^{4,5} Claudin-18 is highly expressed in the airway epithelium; however, stimulation in response to IL-13 decreases claudin-18 expressions

in the airway epithelium.³⁰ In addition, claudin-18-deficient mice with asthma exhibit an increased susceptibility to airway hypersensitivity, suggesting an association with the pathophysiology of asthma.³⁰

A substantial number of factors, the so-called asthma-aggravating factors, can disrupt the barrier functions of the airway epithelium. Therefore, the involvement of airway inflammation in disrupting the epithelial barrier function and asthma is a topic of great interest (Fig. 1).

Disruption of the latent airway epithelial barrier in patients with asthma

Decreased epithelial barrier function associated with asthma is considered to occur because of the direct action of inflammatory substances and allergens on the epithelial barrier, as described in detail above. However, there have been several recent studies that have reported the possibility of latent barrier function abnormalities in airway epithelial cells of patients with asthma. Xiao *et al.*³¹ cultivated airway epithelial cells obtained from patients with asthma and healthy individuals using the air-liquid interface (ALI) method and observed changes in the epithelial barrier function of differentiated airway epithelial cells. These findings indicated that the disruption of the epithelial barrier function decreased with asthma severity. Furthermore, cultivation of airway epithelial cells using the ALI method under the same cultivation conditions induces the differentiation of airway epithelial precursor cells into airway epithelial cells with cilia after 28 days of cultivation. Thus, Xiao *et al.*³¹ suggested that the fragility of the epithelial barrier of patients with asthma was not because of inflammation only but could also involve pathological features already present in the airway epithelia of patients with asthma. This reduced barrier function can be improved to the same level as healthy individuals by addition of epithelial growth factor (EGF) into the medium, suggesting the potential involvement of EGFR signaling.

Basal cells are airway epithelium precursor cells. The basal cell layer is located below the ciliated columnar epithelium and is positive for cytokeratin 5, cytokeratin 14, and p63. Moreover, basal cells exhibit high EGFR expression levels, which decrease when basal cells differentiate into ciliated epithelium and goblet cells. A study of airway mucosa biopsies obtained from pediatric patients with asthma confirmed that cytokeratin 5-, cytokeratin 14-, and p63-positive basal cell-like epithelial cells proliferated in the airway epithelium.³² Previous studies have reported that EGFR expression levels increased in the airway epithelia of patients with asthma, suggesting abnormal epithelium differentiation.^{33–35} EGF acts on airway epithelial cells, promotes epithelial barrier functions, and suppresses the disruption of the epithelial barrier caused by cigarette smoke exposure.^{31,36} Disruption of the epithelial barrier function caused by HDM allergen, TGF- β , and cigarette smoke exposure can be suppressed using an EGFR inhibitor, suggesting that excessive activation of EGFR is a factor that contributes to a reduced epithelial barrier function.³⁷ Proteins in the Erb family [e.g., EGFR (erb1), Erb2, and Erb3] form homo- and heteroreceptors. Moreover, a knockdown of Erb3 receptors in airway epithelial cells suppresses the reinforcing effect of heregulin on the epithelial barrier.³⁸ Because Erb3 receptors are a death-kinase variant, they are believed to be associated with the formation of the epithelial barrier via the formation of heteroreceptors with other Erb receptors.^{38,39} These differences in Erb receptor signaling could be related to the different formative and disruptive effects of EGFR on the epithelial barrier (Fig. 1).

Destructive

Cigarette smoke
Air pollutants; oxidants,
Respiratory virus; rhinovirus, RSV
Pseudomonas aeruginosa
Protease allergens; Der-p, Der-f1, fungal
Inflammatory cytokines; TNF- α , IFN- γ
Th2 cytokines; IL-4, IL-13,
IL-17 cytokines; IL-17, IL-22, IL-26
Pseudomonas aeruginosa
TLR ligands; LPS, dsRNA

Protective

Glucocorticoids ; budesonide, fluticasone
 β 2-adrenergic receptor agonist
1,25-Dihydroxyvitamin D3
Growth factors; EGF, heregulin
Fatty acid metabolites; PUFAs, lipoxinA4
TLR ligands; CpG-ODN

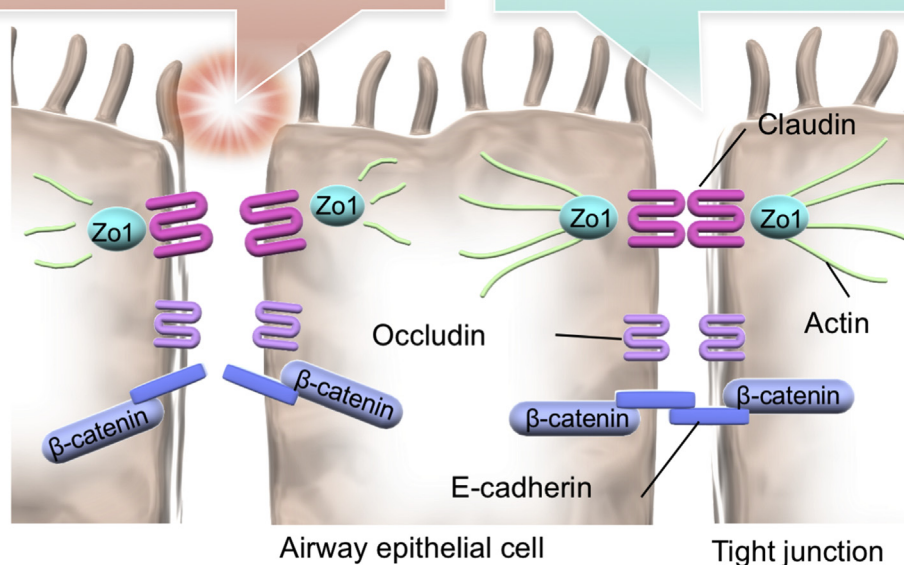


Fig. 1. Destructive and protective factors associated with the airway epithelial barrier.

Association between the activation of the airway epithelium and barrier disruption

The pathophysiology of airway inflammation associated with asthma has been previously explained by eosinophilic inflammation induced by the excessive release of Th2 cytokines by Th2 cells and dendritic cell activation by allergens. A new Th2 cytokine-producing cell, termed group 2 innate lymphoid cells (ILC2), has recently been attracting attention.^{40–42} ILC2s produce large quantities of IL-5 and IL-13 in response to thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, causing pathophysiological changes such as eosinophilic infiltration in the airway and the formation of goblet cells in the airway epithelium.^{7,43} ILC2s also induce IgE production and are considered to induce the Th2 response by activating local dendritic cells.⁴⁴ The fact that ILC2s are in close proximity with the airway epithelium suggests that they play an important role in initiating immediate allergic responses to allergens from the external environment that come into contact with the surface of the airway epithelium.

Currently, much remains unknown regarding the mechanisms by which TSLP, IL-25, and IL-33 are produced by airway epithelial cells. These cytokines are considered to be present in normal nuclei and are released outside the cell when the cell undergoes necrosis.⁴⁵ Apoptotic cells are cut off by caspase-3 and caspase-7 and thus are unable to bind to suppression of tumorigenicity 2 (ST2) receptors. Therefore, these cells lose their ability to induce inflammation. A histological study of IL-33 expression in the airway

epithelium of patients with asthma revealed that the primary cell type that expresses IL-33 was airway epithelial cells.⁴⁶ However, the state in which these airway epithelial cells are in when necrosis occurs remains unknown. If we assume that IL-33 is an important factor associated with triggering allergic inflammation, then there exists a mechanism by which the active types of IL-33 other than those involved in necrosis are secreted by airway epithelial cells. A recent study reported that an alternative IL-33-spliced variant functions to stimulate ST2 and may even be secreted by cells not undergoing necrosis.⁴⁷ There is great interest in how this process is controlled.

Viral infections of the airway reportedly activate epithelial cells, resulting in TSLP and IL-25 production.^{48,49} Virus-derived dsRNA induces TSLP production by airway epithelial cells via TLR3.^{50,51} Viral infections, as mentioned above, disrupt the tight junctions of airway epithelial cells, thereby disrupting the epithelial barrier functions. The TIR-domain-containing adapter-inducing interferon- β (TRIF) pathway via TLR3 activation is important to this process.^{50,51} The myeloid differentiation primary response gene 88 (MyD88) pathway may have a protective effect against barrier disruption caused by dsRNA.⁵¹ Even in the presence of TLR3 stimulation, TLR9 stimulation in response to unmethylated CpG promotes the formation of the airway epithelial barrier, and this effect is mediated by MyD88 activation^{52,53} (Fig. 1, 2).

HDM can potentially cause a similar disruption of the airway epithelial barrier and activation of the epithelium. Long-form-TSLP is reportedly induced following stimulation with HDM. TSLP has

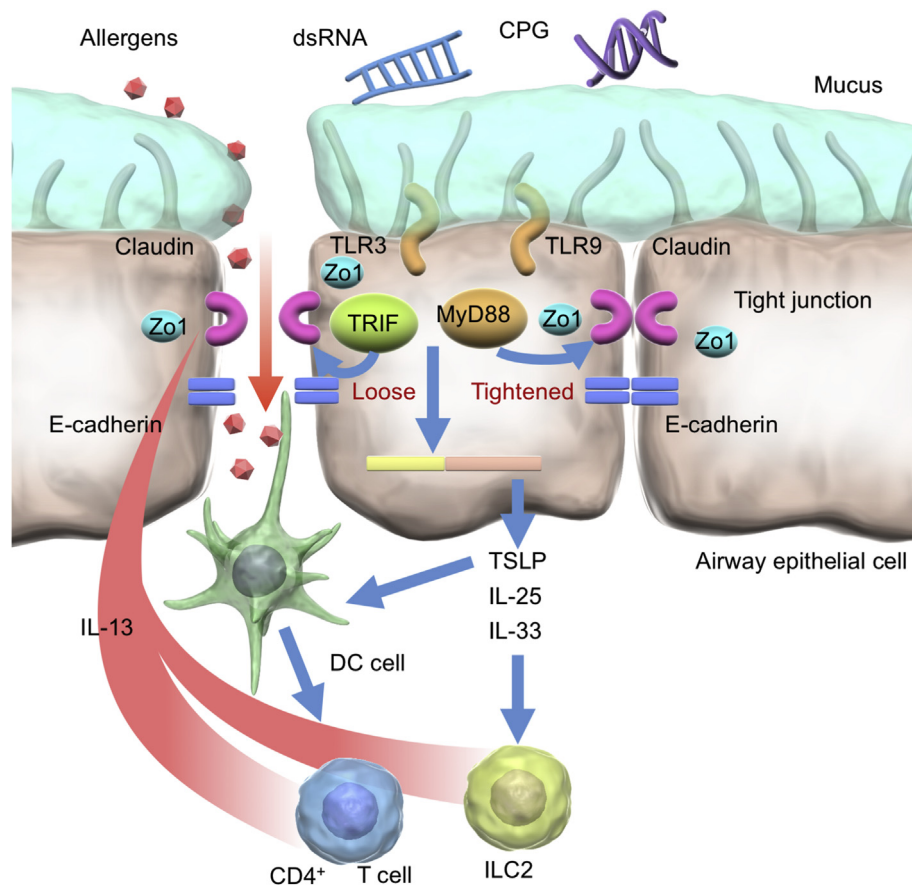


Fig. 2. The role of TLR-3 and -9 in disrupting the barrier functions and activating airway epithelial cells. When dsRNA stimulates TLR3 expressed in airway epithelial cells, the signal disrupts the epithelial barrier via TRIF, and MyD88 signaling conversely acts to protect the barrier. TSLP and IL-25 production via the mediation of TLR3 activates dendritic cells in the submucosa and ILC2s. Thus, IL-13 production, induced by Th2 cells and ILC2s, suppresses epithelial barrier functions.

two isoforms: long-form-TSLP comprising 159 amino acids and a short-form-TSLP comprising 63 amino acids, which includes the C terminus. As mentioned above, the HDM allergen breaks down the tight junction proteins via the action of cysteine protease, thereby disrupting the airway epithelial barrier. Moreover, long-form-TSLP induced by HDM exposure acts on the airway epithelial cells to further disrupt epithelial cell barrier functions via signal transducer and activator of transcription 5 (STAT5) activation.⁵⁴

The activation of ILC2s disrupts the epithelial barrier. Models of the eosinophilic airway inflammation in which IL-33 is administered in the airways of ILC2 knockout mice did not lead to a reduced expression of tight junction proteins because of IL-33 administration or leakage of α -2-macroglobulin and transferrin in the sputum of ILC2 knockout mice.⁵⁵ When the normal human airway epithelial cells and ILC2s are cultivated together, the airway epithelial barrier is disrupted. The fact that the disruption of the airway epithelial barrier caused by ILC2 is suppressed when anti-IL-13 antibodies are present suggests that IL-13 produced by ILC2s in response to IL-33 stimulation is involved⁵⁵ (Fig. 2).

Association between asthma-related genes and epithelial barrier functions

A genome-wide association study identified various genes associated with the suppression of inflammation, including Th2-type immune response characterized by IL-33, ST2, TSLP, ORMDL3, and ADAM33, as disease susceptibility genes.⁵⁶ Among asthma-related disease susceptibility genes already identified,

there were some genes accounted with epithelial barrier functions, although the number of these genes was small.

Multiple studies have demonstrated that the Kinesin Family Member 3A (KIF3A) gene locus is associated with both asthma and atopic dermatitis.⁵⁷ The Kinesin superfamily protein KIF functions as a molecular motor that transports molecules along microtubules.⁵⁸ KIF3A has an important endothelial cell ciliary function in addition to its role as a molecular motor.⁵⁹ Mice that are genetically deficient in KIF3A experience embryonic death; however, a KIF3A deficiency specific to the airway epithelium in mice increases Th2-type inflammation of the airway caused by exposure to allergens (e.g., *Aspergillus* and HDM) and promotes airway hypersensitivity. This is another factor that leads to epithelial barrier dysfunction in addition to disrupted epithelial ciliary motility disorder caused by KIF3A deficiency.⁵⁷

The cadherin-related family member 3 (CDHR3) gene has been identified to be associated with the severe exacerbation of pediatric asthma.^{60,61} CDHR3 is an RV-C receptor related to the exacerbation of asthma severity. CDHR3 is considered to play a role in the stabilization of allogeneic cell junctions.⁶²

In 2009, Koppelman *et al.*⁶³ reported that protocadherin 1 (PCDH1) is a receptor gene for airway hypersensitivity and asthma. PCDH1 is highly expressed in the airway epithelium and has two isoforms: isoform-1 (150 kD) and isoform-2 (170 kD), which have different proteins shared in the extracellular and membrane-spanning domains. PCDH1 increases concurrently with the terminal differentiation of the airway epithelium, suggesting a close association with epithelial function.⁶³ The siRNA silencing of

PCDH1 in airway epithelial cells increases epithelial permeability, indicating that PCDH1 is important to the physical barrier of the airway epithelium.⁶⁴

Glucocorticoids regulates epithelium barrier function

Inhaled corticosteroids are the most effective drugs currently used for treating asthma. Glucocorticoids acts on allergic inflammation by regulating the production of cytokines from immune cells and promoting the formation of the airway epithelial barrier.^{65–67} The stabilizing effect exerted by glucocorticoids on the epithelial barrier is considered to occur because of the stabilization of EGFR receptors on epithelial cells.⁶⁷

Fluticasone and budesonide are synthetic glucocorticoids widely used clinically, and they exhibit a concentration-dependent effect on prompting airway epithelial barrier functions.⁶⁴ Heijink *et al.*⁶⁸ observed that although cigarette smoke exposure inhibited the formation of the epithelial barrier via EGFR phosphorylation, budesonide inhibited the disruption of the epithelial barrier induced by cigarette smoke; this effect was not observed when fluticasone was used.⁶⁸ This difference in the effects that budesonide and fluticasone exhibit is considered to be a result of the differences on their respective effects on EGFR activation and glycogen synthase kinase 3 (GSK-3 β), which is located downstream.⁶⁸ Steelant *et al.*⁶⁹ reported that fluticasone suppressed the dysfunction of the airway epithelial cell barrier induced by HDM exposure and effectively suppressed the acceleration of mucous membrane permeability in animal models. Some genes that are upregulated in the airway epithelium in response to glucocorticoids are related to the effect of glucocorticoids on the stabilization of epithelial barrier functions. PCDH1 is an epithelial barrier regulatory factor and exhibits increased expression in the airway epithelium in response to glucocorticoid stimulation.⁶⁵ NF-E2 related factor 2 (Nrf2) is a transcription factor that guides various antioxidant molecules and protects cells from environmental stressors^{70–72} and is upregulated in the airway epithelial cells in response to glucocorticoids.⁷² Nrf2 was previously believed to play a protective role against oxidative stress induced by exposure to cigarette smoke and other stimulants; however, it may also be related to the stabilization of the epithelial barrier by glucocorticoids.⁷³

The steroid-acute regulatory protein-related lipid transport (START) domain contains approximately 210 amino acids that bind to lipid molecules between the donor and acceptor membranes that transport them. START domains are classified into four protein subfamilies (e.g., ceramide and phosphatidylcholine) that transport different lipids. Moreover, 15 mammalian proteins, including the START domain, have been identified. START domain (Stard) 7 plays a role in transporting phosphatidylcholine to the mitochondria, and interestingly, a Stard7 haploinsufficiency promotes epithelial barrier permeability and markedly exacerbates allergic airway inflammation in mice.³⁸ This effect is related to the fact that a Stard7 deficiency increases oxidative stress and disrupts the epithelial barrier, which is in turn related to mitochondrial DNA damage.⁷⁴

Conclusions

The development of molecular-targeted therapy will allow for further control of eosinophilic airway inflammation. However, asthma continues to worsen in patients with airways that have lost eosinophils, with airway hypersensitivity persisting in many cases. Inflammation is an important factor in the pathophysiology of asthma, but it is difficult to explain tissue-specific factors of asthma by inflammation alone. Asthma has long been considered as an epithelial disease; therefore, to elucidate the role of epithelial

barrier functions on the pathophysiology of asthma, the unknown mechanisms that enhance the sensitivity of the airway to environmental factors should be assessed. Thus, novel therapeutic strategies should be developed in the future.

Conflict of interest

The authors have no conflict of interest to declare.

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